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Hematologic and Immunologic Effects of Extremely Low Frequency (ELF) Electromagnetic Fields

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HEMATOLOGIC AND IMMUNOLOGIC EFFECTS OF EXTREMELY LOW FREQUENCY (ELF) ELECTROMAGNETIC FIELDS

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16. ABSTRACT

This paper critically reviews the ELF literature from 1978 to the present in regard to research dealing with the hematopoietic and immune systems.

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INTRODUCTION

Over the past several years a substantial research effort has been mounted in an attempt to determine what effects, if any, extremely low frequency (ELF) fields have on biological systems. For the most part, this research has been motivated by a growing concern, within the scientific community and consequently the public sector, for potential health and environmental effects associated with ELF exposure. This concern was originally fostered by reports from the Soviet literature dealing with ELF-induced biological effects. More recently, as public awareness of this research area has increased, concern has mounted because of the proliferation of ELF emitting devices which have accompanied technological advancements in this country. In addition to concern for 60-Hz high voltage generating, storage and transmission systems, one of the major underlying motivations for increased concerns about potential ELF biological effects stems from the U.S. Navy's attempt to develop and deploy a submarine communication system which has the potential for large scale environmental exposure to ELF.

A survey of the literature indicates that a substantial effort has been made in response to this concern. Several reviews have indicated a link between ELF and behavioral, neurological and physiological alterations, as well as cellular and molecular interactions (Anderson and Phillips, 1984; Bridges and Preache, 1981; Sheppard, 1983; Waskass, 1981; Tenforde, 1984; Marino and Becker, 1977). Unfortunately, much of the earlier (pre 1977) and some of the current research on ELF biological effects have resulted in inconclusive data with inappropriate interpretation of the results (Bridges and Preache, 1981). Consequently, a review of the ELF literature requires that careful attention be paid to the experimental design of the reported

studies; the ELF exposure system design and exposure regimen; and the statistical analysis of the data in order to avoid arriving at spurious conclusions (Anderson and Phillips, 1984).

The purpose of this paper is to review critically the reported effects of ELF fields on the hematologic and immunologic systems of laboratory animals. Reports dating from 1978 to the present will be evaluated for ELF-induced effects on the cellular and humoral components of these systems during or following *in vivo* or *in vitro* exposure. Reference to earlier reports will be made as appropriate. On the whole, direct comparisons between many of these studies are difficult to make because of differences in species, in exposure parameters, and in the biological endpoints examined. Furthermore, the reported alterations, if present, are in general inconsistent and highly variable, with no apparent trends. Nevertheless, from this review the following generalizations may be formulated: 1) exposure of animals to ELF fields may lead to a variety of changes in the hematologic and/or immune systems; 2) these changes are generally transient and reversible with time; 3) some of the earlier (pre 1978) reported effects of ELF fields on the hematologic and immune systems were probably due to secondary electric field effects (e.g., shocks, noise, vibration, and ozone) which resulted in stress; 4) *in vitro* studies of the reversible alteration of lymphocyte function during exposure to amplitude modulated radiofrequency (RF) radiation are very interesting but require corroborative evidence from both *in vitro* and *in vivo* studies; and 5) there is presently no convincing evidence from *in vivo* and/or *in vitro* experimental studies for adverse alterations in the hematopoietic or immune systems following ELF field exposures.

For convenience, this paper is divided into two general topics:

hematologic effects and immunologic effects of ELF-fields. The latter topic is further subdivided into reviews of studies in which cellular components of the immune system have been exposed in vitro and studies dealing with in vivo exposure to ELF fields. This format is similar to that used in a recent review of the hematologic and immunologic effects of RF radiation (Smialowicz, 1984).

HEMATOLOGY

Hematology is the study of the anatomy, physiology, and pathology of the blood and blood-forming tissues. The hematopoietic system is comprised of a variety of cells and cell products. In fetal life, the production of blood cells occurs in the liver, spleen, and bone marrow. After birth this function is limited largely to the bone marrow, which produces red cells (erythrocytes), white cells (neutrophilic, eosinophilic, and basophilic granulocytes; lymphocytes; and monocytes), and platelets. Each of these cell types performs specific functions that are essential to life. For example, mature erythrocytes transport O_2 to tissues, granulocytes and monocytes phagocytize invading microorganisms, and lymphocytes are involved in immune responses. These functional cells are all descendants of progenitors (stem cells) that reside within the bone marrow. Blood-cell formation consists of two essential processes, proliferation and differentiation; bone marrow progenitor cells proliferate and differentiate into red and white cells. As the process of differentiation progresses, the capacity for cellular proliferation decreases. Impairment of either of these processes may lead to dysfunctions in the hematologic system.

Most reports of ELF-induced changes in the hematopoietic system have focused on the overall distribution and number of the cellular components

in peripheral blood. In a review of the pre-1978 literature Bridges and Preache (1981) indicated that one of the most frequently affected hematological variables in animals exposed to ELF fields was the distribution of leukocytes in peripheral blood. However, until recently, little attention has been paid to the underlying causes or potential functional changes associated with these observations. It is important to emphasize that the hematopoietic, and for that matter the immune system, function as multicomponent systems in which no one component functions mutually exclusive of the others. Furthermore, the hematopoietic and immune systems are affected by other physiological systems which can exert their influence either directly or indirectly.

Table 1 summarizes the post-1977 literature on the hematologic effects of ELF fields in experimental animals. While changes in leukocyte and erythrocyte numbers and distribution have been reported, there is no general trend or consistency within or across studies.

Ragan et al. (1979), in a well designed group of studies, were unable to identify any unequivocal hematologic effects in rats and mice exposed to 60-Hz ELF fields at unperturbed field strengths of 100 kV/m (rats) and 0.15-0.25 kV/m (mice) for 21 hr/day for 15, 30, 60 or 120 consecutive days. While statistically significant differences between exposed and sham-exposed rats and mice were observed in initial studies, these investigators failed to replicate these effects in subsequent experiments. For example, following a 60-day exposure, a significant decrease was observed in circulating neutrophils and lymphocytes from exposed mice. However, in a replicate experiment just the opposite effect was observed, that is an increase in neutrophil and lymphocyte counts. When these replicate experiments were combined, no

Table 1. Summary of Studies of Hematologic Effects of ELF Fields

Effects	Species	Exposure Conditions				Reference
		Fields	Frequency	Field Strength	Duration (day x hr)	
No consistent, statistically significant and reproducible differences in hematologic parameters except an increase in platelet concentration in rats after 60 days.	Rats	Electric	60-Hz	100 kV/m	15,30,60 or 120 x 21	Ragan et al. (1979)
	Mice	Electric	60-Hz	0.15-0.25 kV/m		
No consistent, statistically significant and reproducible differences in hematologic parameters examined.	Rats	Electric	60-Hz	100 kV/m	15,30,60 or 120 x 21	Ragan et al. (1983)
Decrease in leukocyte counts and hemaglobin and increase in juvenile neutrophils in females but not males.	Mice	Electric	60-Hz	240 kV/m	204 x 22	Fam (1980)
Decrease in erythrocyte count and hematocrit.	Mice	Electric	60-Hz	5 kV/m	2 x 24	Marino et al. (1980)
No differences in hematologic parameters.	Rats	Electric	45-Hz	2,10,20,50 or 100 V/m	28 x 24	Mathewson et al.(1979)
Increases in neutrophil and lymphocyte counts.	Rats	Electric	50-Hz	100 kV/m	56 x .5 14,35 or 49 x 8	Cerretelli et al.(1979)
Increases in reticulocyte counts and decrease in hemoglobin concentration.	Dogs	Electric	50-Hz	25 kV/m	14,28 or 42 x 8	Cerretelli et al.(1979)
No differences in hematologic parameters.	Mice	Magnetic (Alternating)	60-Hz	0.11-T	23 x 7	Fam (1981)
No differences in hematologic parameters.	Rats	Magnetic (Rotating)	0.5-Hz	10^{-3},10^{-4} or 5×10^{-6}-T	5 x 24 (Pre- and/or Postnatal)	Presinger et al.(1978)

significant differences were observed between exposed and sham-exposed mice. Similarly, in two replicate experiments in which rats were exposed for 15 days to ELF fields at 100 kV/m, leukocyte levels were reduced in one study but in a subsequent study no reduction was observed.

Application of rigorous statistical evaluations of hematologic data from a series of experiments in which rats were exposed to 60-Hz ELF fields at 100 kV/m for up to 120 days resulted in the failure to detect any consistent effects (Ragan et al., 1983). These data are presumably those presented above (Ragan et al., 1979). These investigators reported that within any individual study it was not unusual to detect significant differences between exposed and sham-exposed animals for certain variables. They, therefore, emphasized the need to design studies in which replicate experiments are employed to insure corroboration of subtle biological effects due to chemical or physical insults of low toxicity and to employ appropriate multivariate analyses (Ragan et al., 1983).

Fam (1980) exposed male and female mice to a 60-Hz field at an unperturbed field strength of 240 kV/m, 22 hr/day, 7 days per week for up to 204 days. No differences were observed between exposed and sham-exposed males for the hematologic parameters examined. However, exposed female mice had significantly lower leukocyte counts and lower hemoglobin concentrations compared with sham-exposed females. The decreased hemoglobin concentrations in exposed females was not accompanied by a concomitant decrease in erythrocyte counts nor a decrease in hematocrit, which calls into question the physiological significance of this decrease in hemoglobin. A significant increase in the relative percentage of band neutrophils was observed in exposed females, however, no other shift in leukocyte populations was

observed. The increase in juvenile neutrophils and the lack of any shift in the other leukocytes from exposed female mice is not consistent with the observed decrease in total leukocyte count. These results are therefore of questionable value since they are physiologically inconsistent. Ragan et al. (1983) pointed out that Fam (1980) employed a univariate t-test to evaluate each hematologic endpoint rather than a more appropriate multivariate analysis. Furthermore, no replication of this study was performed to corroborate the findings in female exposed mice.

Male and female mice were exposed to a 60-Hz, 5 kV/m electric field for 2 days by Marino et al. (1980). Mice were either sham exposed for 2 days followed by a 2 day ELF exposure or vice versa. Two blood samples were obtained from the orbital sinus from each mouse, one after the first 2 day period and the other after the second 2 day period. A consistent significant decrease in erythrocyte concentration was observed in both male and female mice following the second 2 day period regardless of whether the field exposure preceded or followed the nonexposure. A decrease in hematocrit accompanied the decrease in erythrocyte counts in 3 of 4 experiments, but hemoglobin concentrations were decreased in only 2 experiments. No other consistent changes were observed in erythrocyte parameters examined. Marino et al. (1980) attributed their results to the ability of the mice to respond physiologically to a change in their electrical environment rather than as a result of the electric field itself. They indicate that the observed changes are consistent with the electric stress hypothesis (Marino et al., 1976). However, Ragan et al. (1983) questioned this conclusion since "corticosteroids have a stimulatory, not depressive, effect on erythropoiesis." Another criticism of this work by Ragan et al. (1983) deals with the fact

that Marino et al. (1980) "treated these highly interdependent parameters as independent variables" in their statistical analysis of the data. In any event, while the measured erythrocyte parameters are somewhat internally consistent, the physiological significance of these findings (Marino et al., 1980) is questionable, particularly in light of the fact that these hematologic values are within normal limits (Mituka and Rawnsley, 1977).

Mathewson et al. (1979) found no consistently reproducible differences between male rats exposed for 28 days (24 hr/day) to 45-Hz vertical electric fields at field intensities of 2, 10, 20, 50 or 100 V/m compared with sham-exposed rats in 3 replicate experiments. In a fourth experiment, in which rats were exposed to 20 V/m for 28 days, no significant differences were observed compared with controls. The hematologic parameters examined were the following: erythrocyte count, hematocrit, hemoglobin concentration, and total and differential leukocyte counts. Furthermore, necropsy and histopathological examination of tissue from 16 organs, including spleen and adrenal gland, revealed no changes that could be attributed to the ELF electric fields.

Cerretelli et al. (1979) exposed rats for periods ranging from 30 min to 8 hr per day for up to 2 months to 50-Hz fields at an intensity of 100 kV/m. These investigators found a significant increase in the percentage of neutrophils and a decrease in lymphocytes in peripheral blood under all exposure conditions. These alterations were reported to persist for up to 7 weeks following the ELF exposure. No difference, however, in the total leukocyte count between exposed and control rats was observed under any of the exposure conditions. The significance of these finds is difficult to determine because the actual data are not presented (changes are indicated

by +, -, or o). Furthermore, the statistical analyses employed to arrive at the indication of increased (+) or decreased(-) cell counts are not provided.

In the same report Cerretelli et al. (1979) present results from experiments in which dogs were exposed for 2 to 7 weeks to 50-Hz fields at intensities of 10 or 25 kV/m. No differences in the hematologic parameters examined were observed in dogs exposed to the 10 kV/m fields compared with controls. A significant increase in reticulocyte counts and a decrease in hemoglobin concentration were observed in dogs exposed for 2 or 4 weeks to 25 kV/m fields. No changes in erythrocyte counts or hematocrits accompanied the reported changes in hemoglobin concentration and reticulocyte counts at these higher field strengths, thereby suggesting that these results are not physiologically consistent. Again, the authors do not present the actual data nor do they indicate the analyses performed. Consequently, the results presented by Cerretelli et al. (1979) are of questionable value for providing a reliable database upon which conclusions about the hematologic effects of ELF fields can be made.

Fam (1981) examined the hematologic effects of exposure of male mice to alternating magnetic fields. Mice were exposed for 23 hr per day for 1 week to 60-Hz, 0.11-T alternating magnetic fields. No differences in erythrocyte or leukocyte parameters were observed between exposed and control mice. It is interesting to note that the leukocyte counts for the control mice in this study were 43% higher than the leukocyte counts from control male mice in the electric field study reported by Fam (1980), i.e., 9.63 x $10^3/mm^3$ and 5.51 x $10^3/mm^3$, respectively. This comparison of data underscores the care that should be taken in evaluating these data. The highly

variable nature of these hematologic parameters is obvious despite the the fact that mice of the same sex and strain (i.e., SW-ICR) were used.

Using low intensity magnetic fields, Persinger et al. (1978) found no significant differences in differential leukocyte counts between exposed and nonexposed rats. Rats were exposed from 2.5 days before birth to 2.5 days after birth to either 0.5-Hz rotating magnetic fields (RFM) at field intensities of 10^{-3}, 10^{-4} or 5×10^{-6}-T or to sham fields. These rats were then exposed as adults to either one of three 0.5-Hz rotating magnetic fields of 10^{-6}, 10^{-7} or 10^{-8}-T or sham fields or room control conditions. Although no differences were observed in leukocyte counts from peripheral blood, a 17% decrease in thymus weights was observed in rats that had been exposed perinatally to the magnetic fields compared with sham-exposed rats. These authors indicated that "despite the small absolute differences between ... thymus weights between perinatal RMF-exposed and sham field-exposed rats, the effects were comparable in magnitude to the differences between types of postweaning caging, a condition sufficient to also significantly alter body weights, consumptive behaviors, and several blood measures". In light of these findings, the physiologic significance of the observed decrease in thymus weight in RMF-exposed rats is highly questionable.

In summary, a variety of changes in hematologic parameters has been reported to result from exposure of experimental animals to electric or magnetic ELF fields. Questions about the internal consistency between the blood parameters measured, the physiological significance of the reported changes, and the appropriateness of the statistical analyses employed have been raised in those studies in which alterations have been reported. Because of the inherent biological variability of these measured parameters, the determination of a "statistically significant" difference between exposed and sham-

exposed animals is not sufficient evidence upon which to conclude that ELF fields alter the hematopoietic system. This fact is underscored by the work of Ragan et al. (1979, 1983) and Mathewson et al. (1979) in which purported significant differences in blood parameters in one experiment were not corroborated in subsequent experiments. Based on the above studies, no strong case has been made for any physiologically significant alteration in the hematopoietic system of experimental animals exposed to ELF fields under the conditions described.

IMMUNOLOGY

The immune system is comprised of myriad mechanical, cellular, and humoral components that act as the body's defense against various pathogenic microorganisms, viruses, and neoplasias. The immune system is broadly divided into the soluble elements, i.e., antibodies (humoral immunity) complement interferon and lymphokines, and the cellular elements, which are composed of the lymphoid and phagocytic cells. The phagocytic cells responsible for engulfing and digesting certain microorganisms are the neutrophils or polymorphonuclear leukocytes (PMNs) and the monocytes or macrophages. In the presence of antibodies and complement, neutrophils are aided in engulfing and digesting invading organisms. The monocyte is also a phagocytic cell. Monocytes move into an area in which an infection has begun and then differentiate into macrophages. Macrophages can be "activated" to kill certain microorganisms (e.g., intracellular, facultative bacteria such as Mycobacteria tuberculosis and Listeria monocytogenes, viruses, and fungi) through the interaction of certain subpopulations of lymphocytes, such as the T lymphocytes (Friedman, 1978).

The other cellular components of the immune system are the lymphocytes.

These cells are broadly divided into two groups, the B lymphocytes and the T lymphocytes. Although these cells are similar morphologically, they are different functionally; B and T lymphocytes can be distinguished by the presence of unique antigens or receptors on their membrane surfaces. Both T and B lymphocytes are believed to originate in the bone marrow and then to proceed through various stages of development and differentiation, maturing into functional cells of the immune system (Benacerraf and Unanue, 1979).

The B lymphocyte, or bursa-equivalent lymphocyte, is responsible for humoral immune responses. The B lymphocytes, after appropriate stimulation by antigens, proliferate and undergo morphological changes and develop into plasma cells that actively synthesize and secrete antibodies (Glick et al., 1956).

The T lymphocyte, or thymus lymphocyte, is processed through the thymus after leaving the bone marrow. Classically, cell-mediated or T lymphocyte responses include protection against viruses, fungi, and several bacteria. T lymphocytes are also involved in reactions such as delayed hypersensitivity or contact hypersensitivity and rejection of tumors and foreign tissues such as transplants (allografts). Cell-mediated reactions are so named because these reactions, which operate by specifically sensitized T lymphocytes, can be transferred by these cells to normal animals. B lymphocyte-mediated humoral responses, in contrast, are transferable by serum (Miller et al., 1962).

The recent availability of monoclonal antibodies has made the typing of lymphocyte subpopulations possible on a routine basis. Various functional categories of T cells can be recognized: "helper" (inducer/amplifier) T cells, "suppressor" T cells, and "cytotoxic" T cells. Immature thymocytes may be identified by surface antigens distinct from mature peripheral T cells. Cellular interactions between the various T cell subtypes and other

cells of the immune system are vital to the modulation of the immune response. Because of their pivotal role in enhancing antigenmediated immune responses, disorders involving T-cell subsets may result in immunodeficiency syndromes involving either cell-mediated or humoral immunity (Bellanti and Rocklin, 1979).

Each element of the immune system plays a cooperative role in defending the host against infection and disease. At the same time a delicate balance is maintained which prevents the immune system from reacting to the host tissues, thus preventing autoimmune reactions. The alteration or dysfunction of any of these elements may lessen the host's ability to combat infection or may lead to autoimmune disease. However, because of adaptability and redundancy in the immune system, the host can generally survive subtle perturbations. Consequently, although subtle effects on the immune system may be generated by physical or chemical agents, all such effects may not lead to clinically significant immune dysfunctions.

In addition to being internally regulated, the immune system is responsive to other physiological systems of the host. The endocrine system has a major influence on the distribution and function of immune cells (Ahlqvist, 1981). A number of studies have shown that changes in circulating glucocorticosteroid levels, due to various types of stress, cause major shifts in distribution of different cell populations of the immune system as well as changes in function (Daugherty and Frank, 1953; Claman, 1972; Fauci, 1975; Schultz et al. 1979; Riley, 1981). Similarly, it is now apparent that the nervous system imposes some control over the immune system (Ader, 1981). Exposure of animals to stress, such as intermittent foot shock, causes the release of opioid peptides from central and peripheral sites (Shavit et al.,

1984). These peptides have been shown to alter the distribution and function of several immune cells including T cells, NK cells, and granulocytes (Gilman et al., 1982; Mathews et al., 1983; Wybran et al., 1979).

Thus, any consideration of the immunological bioeffects of external stress such as ELF must deal with potential indirect effects such as those mediated by glucocorticosteroids and opioid peptides as well as direct effects on components of the immune system itself.

In vivo Studies

Table 2 summarizes the immunologic effects of exposure of experimental animals to ELF fields. Cerretelli et al. (1979) summarizes the effect of electric fields on resistance to infection in mice as reported by Margonato, Viola and Cantone, at the Congress of the Italian Physiological Society in 1978. Male and female mice were exposed for 8 hr/day for 42 days to 50-Hz electric fields at a field strength of 25 kV/m. The mice were injected intraperitoneally with graded concentrations of Staphylococcus pyogenes (aureus Smith var.) following exposure. Resistance to infection was checked against identically injected control mice. The $LD_{50(72)}$ was found to be the same in exposed and control mice. Unfortunately, these results are impossible to evaluate because the actual data are not presented by Cerretelli et al. (1979). In earlier work, Krueger and Reid (1975) failed to observe any change in the resistance of mice exposed to influenza virus following exposure for 21 days to 75-Hz ELF at 100 V/m.

In two well designed and executed studies, Morris and Ragan (1979) and Morris and Phillips (1982) were unable to detect any differences between mice exposed to 60-Hz electric fields and sham-exposed mice for a variety of immunological endpoints. Male or female mice exposed for 21 hr per day for

Table 2. Summary of Studies of Immunologic Effects (*In Vivo*) of ELF Fields

Effects	Species	Exposure Conditions				Reference
		Fields	Frequency	Field Strength	Duration (day x hr)	
No change in resistance to infection with *S. pyogenes*.	Mice	Electric	50-Hz	25 kV/m	42 x 8	Cerretelli et al.(1979)
No differences in IgM, IgG or complement, no differences in T- and B-lymphocytes numbers and no differences in antibody response to KLH.	Mice	Electric	60-Hz	0.15-0.25 kV/m	30 or 60 x 21	Morris and Ragan (1979)
No differences in antibody response to KLH and mitogen stimulated responses of T- and B-lymphocytes.	Mice	Electric	60-Hz	0.15-0.25 kV/m	30-150 x 20	Morris and Phillips(1982)
Elevation in mast cells in thymus.	Rats	Magnetic (Rotating)	0.5-Hz	10^{-3},10^{-4} or 5 x 10^{-6}-T	5 x 24 (Perinatal)	Persinger and Coderre (1978)
Decrease in thymus weight.	Rats	Magnetic (Rotating)	0.5-Hz	10^{-3},10^{-4} or 5 x 10^{-6}-T	5 x 24 (Perinatal)	Persinger et al. (1978)
Partial restoration of suppressed antibody response to SRBC in mice held in a Faraday cage.	Mice	Electric	50-Hz	15 kV/m	540 x 24	Fischer et al. (1981)

30 or 60 days to 60-Hz unperturbed electric fields at field strengths of 0.15-0.25 kV/m showed no consistent change compared with sham-exposed mice in the following: 1) IgM, IgG or complement levels; 2) numbers of splenic T and B lymphocytes; 3) primary antibody response to keyhole limpet hemocyanin (KLH); or 4) leukocyte or lymphocyte counts in peripheral blood (Morris and Ragan, 1979). In another study Morris and Phillips (1982) exposed male mice to 60-Hz electric fields at field strengths of 0.15-0.25 kV/m for 20 hr per day for 30-150 days. No significant differences were observed in primary antibody responses to KLH between exposed (30 or 60 days) and control mice. Furthermore, no significant changes in the mitogen stimulated responses of T and B lymphocytes were observed between mice similarly exposed for 90 or 150 days compared with sham-exposed animals.

The above two studies provide strong evidence for the lack of any biologically relevant and consistent statistically significant effects of ELF fields on the humoral or cellular components of the immune system of mice. Furthermore, these studies indicate that the functional integrity of the components of the immune system examined is not affected under the described conditions of ELF exposure.

Persinger and Coderre (1978) reported that adult rats that had been perinatally exposed to rotating magnetic fields (0.5-Hz, 10^{-3} to 10^{-6}-T) displayed a "marginally significant" elevation in thymic mast cell number (20-35%) relative to sham-exposed controls. However, rats exposed as adults only did not show significant changes in the number of mast cells in the thymus. These investigators indicate that the increase in thymic mast cells in perinatally exposed rats correlates with the reduction in thymus weight reported in rats similarly exposed (Persinger et al., 1978). They suggest that these changes in thymic mass and cellularity might be attributed to

the release of glucocorticoid, ostensibly due to a stress reaction, since glucocorticoids induce thymic mastocytopoesis in the rat (Persinger and Coderre, 1978). They feel that the most "impressive feature" of their findings is the "apparent long-term expression of the change". The significance of these results is highly questionable both from a physiological and a statistical standpoint. The thymic mast cells were elevated in 200 day old rats despite the fact that the post weaning treatments varied. Furthermore, the investigators admit to a "large statistical overlap with sham-field controls" and state that the purported change in mast cell members is only "marginally significant" (Presinger and Coderre, 1978).

Fischer et al. (1981) reported that mice placed in a Faraday cage over an 18 month period had significantly reduced primary antibody responses to sheep red blood cells (SRBC) as measured by the hemolytic plaque assay. When mice housed in a Faraday cage were exposed to a 15 kV/m, 50-Hz field, a slight increase was observed in the antibody response compared with Faraday caged mice. Decreased and increased hemagglutination titers accompanied the decreased and increased plaque numbers for Faraday caged mice and mice exposed to 50-Hz fields, respectively. These results were obtained in two replicate experiments. These investigators offer no conclusions about the possible consequences of the observed effects, except that screening ambient atmospheric electric fields appears to suppress the immune response and that the screening or "Faraday effect" can be compensated for by introducing ELF fields. While these are intriguing results much more work is required to corroborate these findings which suggest that 50-Hz fields are beneficial to the immune response of mice.

In summary, the above <u>in vivo</u> studies fail to provide substantial

convincing evidence for physiologically significant and reproducible effects of ELF fields on the immune system of experimental animals under the conditions described.

In vitro Studies

Table 3 summarizes the immunologic effects of in vitro exposure to ELF fields. Conti et al. (1983) exposed human peripheral blood lymphocytes to square-wave magnetic fields at 1, 3, 50 or 200-Hz at field strengths of 23-65 gauss in the presence or absence of mitogens. Lymphocyte cultures were either exposed to ELF fields throughout the 72 hr incubation period or for intermittent periods during the 72 hr culture period. The three mitogens used were concanavalin A (Con A) and phytohemagglutinin (PHA), both T-cell mitogens, and pokeweed mitogen (PWM) which stimulates B-cells as well as T-cells. These investigators reported that exposure of cultures for 72 hr resulted in a significant ($p<0.01$) reduction in PHA stimulated lymphoproliferation at each of the 4 frequencies. Con A-stimulated lymphoproliferation was reduced at 3- and 50-Hz, while PWM responses were affected only at 3-Hz. Unfortunately, we have to take the authors' word for these "significant differences", because there are no indications in the table as to which of the many data sets of ELF exposed vs nonexposed cultures are different. At a frequency of 3-Hz, where significant reductions in PHA, Con A and PWM responses were observed, lymphocytes cultured in the absence of any mitogen and exposed to the magnetic fields had consistently reduced ^{3}H-thymidine incorporation compared with matched control cultures. Because the authors do not indicate in their table of data if any of these "no mitogen" ELF exposed lymphocytes are significantly different from controls, it is difficult to determine if these reductions might contribute to the reductions in mitogen responses at 3-Hz.

Table 3. Summary of Studies of Immunologic Effects (In Vitro) of ELF Fields

Effects	Cells	Exposure Conditions				Reference
		Fields	Frequency	Field Strength	Duration (hr)	
Decrease in PHA, Con A and PWM stimulated ^{3}H-thymidine incorporation.	Human lymphocytes	Electro-magnetic	1,3,50 or 200-Hz	(23-65 G)	12 or 72	Conti et al. (1983)
Decrease in lymphocyte viability with increasing pulse discharge time or increasing field strengths.	Human lymphocytes	High voltage pulses (transient currents)	50-Hz	>2.6 kV/cm	>1us	Mild et al. (1982)
Decreased T-lymphocyte cytotoxicity.	Mouse CTLL-1 cell line	AM/RF	16,40,60, 80 or 100-Hz, 450-MHz	RF=1.5mW/cm^2	4 hr	Lyle et al. (1983)
Decreased protein kinase activity.	Human lymphocytes	AM/RF	16,40 or 60-Hz, 450-MHz	RF=1 mW/cm^2	0.25-0.5	Byus et al. (1984)
No change in capping of B lymphocytes in absence of temperature increase.	Mouse lymphocytes	AM/RF	9,16 or 60-Hz, 147-MHz	RF=0.1-48 mW/cm^2	0.5	Sultan et al. (1983)

In other experiments in this report Conti et al. (1983) indicate that exposure of lymphocytes with 3-Hz ELF fields only during the first 12 hr of culture resulted in significant reductions in ^{3}H-thymidine incorporation in the presence of PHA and Con A but not PWM. When lymphocytes were exposed at 3-Hz for the last 48 hr but not the last 6 hr only of culture, PHA and Con A but not PWM responses were also reduced. The data from these experiments are presented in a figure, which, like the data for the 72 hr ELF field exposures, does not indicate which data sets are "significantly different". Furthermore, it appears from this table that one "no field" control for each mitogen was used for the field exposure during the first 12, last 48 and last 6 hr of culture. The interpretation of these results is difficult not only because of problems in the design of the experiments but also because inappropriate statistical analyses were performed on the data.

Conti et al. (1983) indicate that at the end of the 72 hr culture period, "the cell viability was evaluated by trypan blue exclusion and it was always over 90% both field off and field on", however, these very important data are not presented. Even if the viability data indicated no difference between "field off" and "field on" conditions this does not preclude the possibility that there may have been fewer cells in the "field on" cultures compared with the "field off" cultures. If there were fewer cells in the "field on" cultures this could account for the observed reduction in mitogen responses. Until such evidence is presented which eliminates this possiblity it is premature to make any conclusions about these data. While these results are interesting and suggest that ELF fields may affect lymphocyte responsiveness to mitogens, more thoroughly designed and analyzed experiments are needed in order to corroborate the present data.

Mild et al. (1982) exposed human peripheral blood lymphocytes *in vitro* to electric pulse discharges at pulse lengths of 0.2, 1 and 3 us at field strengths of 2.6 kV/cm. At field strengths above 2.6 kV/cm, pulse discharge times of 1 and 3 us increased the number of trypan blue stained (i.e., non-viable) cells. For a shorter pulse (i.e., 0.2 us) a field strength of about 5 kV/cm was required to cause a decrease in lymphocyte viability. These investigators conclude that cell membrane damage occurs at a critical peak E-field in the sample of about 2.6 kV/m. If one assumes $\sigma = 0.6$ s/m then the current density at this field strength is about 0.16 A/cm^2. This current density is less than that which would be expected if, for example, a spark discharge enters the body of a person at the index finger. These investigators state that it is "therefore reasonable to assume that a spark discharge ... may cause cell damage to the leukocytes also *in vivo*". Whether the damage caused *in vitro* would result *in vivo* is uncertain because of the rapid spreading of the current in the body during the discharge.

There are two recent reports on alterations in lymphocytes exposed *in vitro* with RF radiation sinusoidally amplitude-modulated at ELF frequencies. While these studies did not employ ELF electric or magnetic fields, they are worthy of mention in this review because of their potential importance in electromagnetic radiation biologic effects research.

Lyle et al. (1983) reported a significant inhibition of allogeneic cytotoxicity of mouse lymphoma MPC-11 target cells by the murine CTLL-1 T-lymphocyte effector cell line during a 4 hr cytotoxicity assay conducted in the presence of a 450-MHz RF field amplitude-modulated at 60-Hz. Exposure of the effector cells to the field prior to addition to target cells also resulted in inhibition of cytotoxicity. This inhibition was found to be affected by exposure to the fields for the first 2 hr of the 4 hr culture

period; however, only partial inhibition was observed when the cultures were exposed during the last 2 hr of the culture period. Kinetic studies revealed that the field induced inhibition of cytotoxicity at 450-MHz AM persisted for only 9 hr. Inhibition of cytotoxicity was observed in a series of experiments at AM frequencies of 16-, 40-, 80- and 100-Hz. Inhibition of cytotoxic T-cells at these frequencies was not as dramatic as that produced under 60-Hz exposure. No increases were reported in culture temperatures in exposed compared with control cultures. The incident power density was 1.5 mW/cm^2 during exposures. Cell viabilities were reported to be "99% or greater ... after a 4-hr fi ld exposure", although these data were not presented. No reduction in allogeneic cytotoxicity was observed in the absence of an unmodulated 450-MHz field. This suggests that the amplitude modulation was responsible for the observed effect. It would be interesting to determine what effect, if any, 60-Hz electric and magnetic fields might have on this system.

The results of Lyle et al. (1983) suggest that non-thermogenic RF radiation amplitude-modulated fields affect T-lymphocyte cytotoxic activity in vitro. This work must be corroborated and in vivo studies undertaken before any claims about the physiological significance of these findings can be made.

In other work from this lab, Byus et al. (1984) reported that a decrease in protein kinase activity in human tonsil lymphocytes occurred during 15 and 30 min exposures to a 450-MHz RF field sinusoidally amplitude modulated at 16- or 60-Hz. This reduction in non-cAMP-dependent protein kinase returned to control or preexposure levels by 45 and 60 min. It is interesting to note that the "specific identity of this kinase is unknown". Consequently, the biological role played by this kinase and the

physiological significance of its reduction during RF field exposures are unknown. No change in kinase activity was observed when only 450-MHz continuous wave fields were employed. No differences were observed in viability or culture temperatures between exposed and sham-exposed lymphocyte cultures. These findings are similar to those reported by Lyle et al. (1983) in that they purport to show a "windowing effect with respect to modulation frequency and exposure duration" (Byus et al., 1984). However, since both phenomena are reversible, their in vivo physiological significance is questionable.

Sultan et al. (1983) failed to demonstrate any change in the ability of mouse B lymphocytes to "cap" antigen-antibody complexes on their cell surface during exposure to non-thermogenic levels of 147-MHz RF fields amplitude modulated by 9-, 16- or 60-Hz sine wave when cultured at 37°C. When lymphocytes were cultured at 42°C, however, "capping" was equally inhibited in both control and irradiated cells. No significant differences in capping were observed between exposed and control cells at any modulation frequencies and power densities provided that both preparations were maintained at the same temperature.

The different results reported by Sultan et al. (1983), Lyle et al. (1983) and Byus et al. (1984) are difficult to reconcile. In each case, the studies appear to have been well designed and executed with appropriate statistical analyses applied. Lyle et al. (1983) and Byus et al. (1984) reported alterations in lymphocyte function or biochemistry especially at an AM frequency of 60-Hz, however, Sultan et al. (1983) failed to observe any effects. In each of these three studies the only common thread is the amplitude modulation frequency of 60-Hz. The cells employed and the endpoints

study in each of these three reports were totally different, and one might argue that this is the underlying reason why Sultan et al. (1983) failed to observe any effects. However, Lyle et al. (1983) and Byus et al. (1984) used two totally different cell types (T-lymphocyte cell line and human tonsil lymphocytes, which are comprised of 50% T and 50% B lymphocytes, respectively) and yet a 60-Hz "window" was observed in both of those studies. Furthermore, a 60-Hz "window" was observed by Lyle et al. (1983) and Byus et al. (1984) for two totally different endpoints (i.e., cytotoxicity and protein kinase activity, respectively). Unfortunately, these are the kinds of conflicting reports which have plagued nonionizing electromagnetic radiation research for years. Before any informed conclusions about the physiological significance of these findings can be made, independent, replicate experiments must be performed to corroborate the finds.

CONCLUSIONS

Many of the studies reviewed in this paper are flawed because of poor experimental design and statistical analysis of the data. In those studies which appear to be well designed and adequately analyzed, no clear, consistently reproducible and physiologically significant effects of ELF fields on the hematologic and/or immunologic systems or on their cellular elements have been demonstrated. The reported alterations caused by ELF fields have for the most part been found to be transient and mild in nature. The fact that no consistent effects have been observed even when similar exposure conditions and experimental parameters have been employed indicates that low level ELF field-induced effects are very subtle and consequently may be of little or no physiological significance as regards the hematopoietic and immunologic systems.

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